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MICROBIAL ENTOMOPATHOGENS IN CONTROL OF MOSQUITO BORNE DISEASES

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ABSTRACT

Mosquito is the most significant vector in medical entomology. Various species of mosquitoes are implicated in the transmission of a wide variety of human and animal diseases. With the widespread use of chemical insecticides to reduce the burden of mosquito-borne diseases, there is increasing concern of environmental adulteration and its deleterious effects on human health. Consequently, biological methods have gained more importance. Several novel biological control measures are in the process of development. This review provides a brief update of new and existing methods of mosquito control using entomopathogenic microbes.

KEYWORDS: **Entomopathogen, *Wolbachia, Lagenidium*, mosquito-borne diseases**

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INTRODUCTION

Mosquito play an essential role in pathogenesis and the spread of malaria, lymphatic filariasis and Arbo viral infections. Around 207 million malaria cases and 627 000 deaths have been reported worldwide during 2012.[1] The seminal role of vector control has been recognized universally and biological control of mosquito is in the focus of current research. Although chemical insecticides were used earlier extensively in an attempt to reduce the burden of mosquito-borne diseases, it was found to cause deliterious effects on environment resulting in alteration of normal ecosystem.[2] Another major concern was emergence of resistance to these chemical agents among various species of mosquito on continuous use.[3] Consequently, biological methods came up as a ecofriendly vector control approach. Various biological methods have been introduced and several more novel methods are in the process of development. Naturally occuring microbes pathogenic to mosquito larva or adults have been a rich source of biological vector control agents. In this review, the various entomopathogenic microbes and their role the role in control of mosquito-borne diseases have been discussed.

ROLE OF MOSQUITO AS A VECTOR

Among 3400 species of mosquitoes, only few species Anopheles, Culex and Aedes have been primarily implicated in the transmission of human pathogens and have medical importance.[4] Mosquito serves as vector for wide variety of parasitic and viral diseases. This includes malaria, lymphatic filariasis, dengue, chikungunya, yellow fever Rift Valley fever and Arbo viral encephalitis (i.e. Eastern Equine Encephalitis, Western Equine Encephalitis, Venezuelan Equine Encephalitis, St. Louis encephalitis, LaCrosse encephalitis and Japanese B encephalitis).[5, 6] Malaria is the most significant mosquito-borne illness and has global concerns. The disease burden of malaria is not limited to only the sporadic cases and epidemics in endemic region. It is also an important consideration for travellers from non-endemic countries as imported cases of malaria frequently contributes to additional stress on health care system. The resurgence of mosquito-borne diseases in recent years are partly related to insecticide resistance and human activities.[7] The movement of human population, ecosystems changes like urbanization, deforestation, inadequate water and waste management along with climate change have played an imperative role.[7, 8]

BIOLOGICAL METHODS IN VECTOR CONTROL

Aquatic organisms like dragonfly nymphs, cyclopoid copepods, malacostracans, anostracans, tadpoles and larvivorous fish have long been known as natural predators of mosquito larva and were employed as biocontrol measure.[9] In addition, various microbes were also found to have natural pathogenic potential in mosquitoes. This approach mostly precludes the limitations of chemical insecticides i.e. resistance development and environmental pollution. An ideal biocontrol method should be self-propagative (i.e. precludes the need for readministration), specific (i.e. without any deliterious effects on human as well as other organisms in the ecosystem) and should have high infection and mortality rate in most medically important mosquito species. In addition, the entomopathogenic microbes should be easy to culture, maintain and store in laboratory and convient for field administration. Currently no entomopathogen have been found to satisfy all these criteria. However, there is an increasing interest in developing effective bio-control method using advanced technologies such as recombinant DNA technology to widened its scope.

ENTOMOPATHOGENIC BACTERIA

Entomopathogenic bacteria are capable of causing infection in arthropod hosts. *Wolbachia* is a novel endosymbiotic intracellular bacterium widely prevalent among various arthropods. Owing to its presence in egg cytoplasm, it is transmitted vertically and has ability to manipulate the reproduction of host reproduction, such as, feminization, male killing, parthenogenesis and cytoplasmic incompatibility (CI) (Table 1).[10]

Table 1
Mechanism of action of different entomopathogenic bacteria

Bacteria	Mechanism
Wolbachia spp.	Reproductive disorders Life-shortening Interfere with other pathogens Direct inhibitory effect on Plasmodium
*B.thuringiensis*subsp.*israelensis*(Bti)	Mosquitocidal toxins: Cyt1A, Cry4A, Cry4B and Cry11A
B. sphaericus (Bs)	Mosquitocidal binary toxin (Bin), Mtx toxins
B. thuringiensis subsp. *morrisoni*	Mosquitocidal toxins:Cyt1A, Cry4A, Cry4B, Cry11A and a 144-kDa novel Cry1 protein
B. thuringiensis subsp. *jegathesan*	Mosquitocidal toxin: Cry11B
B. thuringiensis subsp. *medellin*	Mosquitocidal toxins
Clostridium bifermentans	Mosquitocidal toxins

CI selectively favours reproduction of infected females and thus ensures the invasion and maintenance of self-propagation of *Wolbachia species* in a wild vector population. It also decreases the lifespan of adult mosquitoes. Since *Plasmodium species* require 8-21 days of extrinsic incubation period to develop into sporozoites infectious to human, curtailing lifespan of the mosquito reduce the risk for malaria transmission to a great extent.[10] Additionally, *Wolbachia* infection imparts resistance in the insect host against a variety of human pathogens which may be related to competition for essential nutrients. Evidence from current research suggests that it has direct inhibitory effect on *Plasmodium*. Transient introduction of *Wolbachia* strain in *Plasmodium* infected adult mosquito has been reported to reduce the mean parasitic levels up to 75-84%.[11] However, the major limitation of this approach in malaria control is the absence of natural vertical transmission of *Wolbachia* infection in *Anopheles* mosquitoes possibly because of its inability to infect the ovaries.[10] Stable transinfection of *Wolbachia* following adaptation in *Anopheles* cell lines and achieving high maternal transmission rate with CI phenotype are the strategies currently under research.

BACTERIAL TOXINS

Another aspect of biological control of mosquito is bacterial insecticides. Unlike *Wolbachia* which targets the adult mosquito, mosquitocidal toxin producing bacteria are designed to control larvae. *Bacillus thuringiensis* subsp. *israelensis* (Bti) and *B.sphaericus* (BS) strains produce toxic proteins such as Cyt1A, Cry4A, Cry4B, Cry11A and binary toxin (Bin) during sporulation.[12] These toxins ingested by mosquito larvae enter the mid gut cells (receptor mediated) and lead to cytolysis. Bs toxin has a narrow spectrum of activity (not active against black flies and some *Aedes species*). Moreover, resistance to Bs toxin among *Culex species* has been reported from India, China, Brazil and France.[12] On the contrary, pertaining to the synergistic action of various Cyt and Cry proteins, Bti toxin displays lethal effects in wide population of mosquitoes and flies without development of resistance. Genetic engineering to develop recombinant bacteria with enhanced expression of both Bti/Bs toxins is of great concern in vector control. These recombinants have shown several fold increase in toxicity, wider spectrum of activity and lesser risk of emergence of resistance.[13]

FUNGAL ENTOMOPATHOGENS

Fungi belonging to Oomycota (e.g. *Lagenidium, Leptolegnia, Pythium* and *Crypticola*), Chytridiomycota (e.g. *Coelomomyces*), Zygomycota (e.g. *Entomophthora* and *Conidiobolus*) and Deuteromycota (e.g. *Culicinomyces, Beauveria* and *Metarhizium*) phyla have reported to parasitize mosquito larvae and cause epizotics in mosquito population.[14] Most of these fungi are aquatic organisms and are facultative parasites of mosquito larvae. Among various watermolds, *Lagenidium giganteum* is foremost important species. Its entomopathogenic role has been investigated extensively and it is the only fungi that has been marketed as mosquito bio-control agent.[14] Both oospores and zoospores of *L. giganteum* induce infection with high mortality in most medically important mosquitoes (*Anopheles*, *Culex* and *Ades species*).[15] However, oospores being more resistant to desiccation and mechanical stress with longer stability in storage conditions have greater implications in large-scale mosquito control programs in comparison to delicate and short-living zoospores. The propagation of this fungi in preferred environmental niche (i.e. stagnant water) allows recurrent infection of several mosquito generations, providing significant operational advantage over entomopathogenic bacterial toxins and chemical insecticides. Similar to *Lagenidium, Coelomomyces* also persists stagnant water of rice fields and cause periodic epizootics in several generations of mosquitoes.[16] However, its host range is mainly restricted to anopheline species. The extent of epizootics are more severe which achieves 50% or more mortality. The difficulty in mass production has limited its use.[16] *Culicinomyces* was found to infect *A. hilli, C. quinquefasciatus*, and *A. aegypti*.[14] Greater activity was observed against *A. aegypti*, an important vector implicated in transmission of dengue and yellow fever. However, the variability of infection rate and mortality, requirement for higher doses and instability in high salinity in storage conditions are the potential drawbacks for its use in large-scale field applications. *Beauveria* and *Metarhizium* have cosmopolitan distribution. While *Beauveria* has a very wide host range, *Metarhizium* infects mainly soil-dwelling insects and few mosquito species.[17] Both fungus produce mycotoxins which are implicated in killing of the insect host. Beauvericin, Beauverolides, Bassianolide, Bassianin, Tenellin and Oosporein are the toxins isolated from *Beauveria sp.* and Destruxins, Swainsinone, and Cytochalasin C are from *Metarhizium sp*.[17-19] However, their use may have adverse effects on human health. *Beauveria bassiana* has been reported to cause sensitivity in human on repeated and massive exposure.[17] In contrast to other fungal entomopathogens, *Entomophthora* primarily infects the adult mosquitoes and has no significant lethal action on mosquito larva.[20] This fungus has several limitations as mosquito control agent. The spores are delicate in nature and lose its potency in humidities below 75%. Although the resting spores have more stability and viability, these are unreliable because of their prolonged dormancy and asynchronous germination.[14, 20]

HELMINTHS - ENTOMOPATHOGENIC NEMATODES

Romanomermis iyengari and *Strelkovimermis spiculatus* are two foremost important mermithid nematode that naturally parasitize larval forms of various species of mosquitos.[21] They penetrate lateral thorax and / or abdominal part of mosquito larva. The emergence of these parasites out of mosquito by penetration results in death of the host. In case of superparasitism, synchronous emergence usually initiated by females in *S. spiculatus* and males in *R. iyengari*.[21] Becnel *et al*. studied the entomopathogenic activity of *S. spiculatus* in nine common mosquito (*Anopheles, Aedes, Culex* and *Toxorhynchites*) species and several aquatic organisms.[22] *S. spiculatus* showed no parasitism in non-target aquatic organisms. Although all species of mosquitoes tested were susceptible, *C. pipiens quinquefasciatus* showed greatest tolerance in terms of lowest initial invasion and mortality. In contrast, *A. taeniorhynchus* and *A. albopictus* were highly susceptible. In another study, the prevalence of *S. spiculatus* infection in mosquito population in the natural habitats ranged from 11% to 100%, with a higher occurrence observed in the months of winter and spring.[23] Among eight species of mosquito larvae sampled from grassy-pool habitats filled with rainwater, six were infected with the *S. spiculatus*. *A. albifasciatus*, a predominant mosquito species in the grassy-pool habitat, showed highest levels of parasitism.[23]

ENTOMOPATHOGENIC VIRUSES

Baculoviruses, densoviruses, iridoviruses and cytoplasmic polyhedrosis viruses accounts for most of the viruses that infect mosquitoes and show the prospect of developing as mosquito control agent.[24] Although the discovery of Mosquito iridescent virus (MIV) in *A. taeniorhynchus* by Clark *et al.* raised considerable interest on its use in mosquito bio-control, its practical difficulties were found in subsequent studies.[25] Peritrophic membrane, especially in first- and second-instar mosquito larvae, serves as an efficient physical barrier which blocks the passage of various viruses out of the lumen of alimentary canal.[26] Consequently, MIV failed to cause a high level infection even if ingested in large amounts by first- and second-instar larvae. It was found that most virus particles were retained and degraded in the midgut abruptly. Although MIV and other virus particles may undergo cellular uptake in the anterior midgut, it was not found to occur at detectable frequency.[26] A subset of Baculoviruses known as Nucleopolyhedro virus (NPV) has long been known to parasitize arthropod insects. An entomopathogenic nucleopolyhedro virus originally isolated from Culex nigripalpus (CuniNPV) was found to cause infection exclusively in Culex mosquito species.[27] While other mosquito genera and *C. territans* remain resistant to it, CuniNPV has shown promising results in *C. quinquefasciatus* and *C. nigripalpus* larvae. The virus particles (occlusion bodies) ingested by mosquito larvae multiply in the gastric caecae and midgut epithelial cells producing expansion and opacity in nuclei of as the first sign of infection within 24 to 28 hours. The infected cells soon become leaden with viral occlusion bodies. CuniNPV infection leads to stunted development of mosquito larvae and ultimately it results in death of the larvae within 72–96 hours.[28] Occlusion bodies released in aquatic habitat initiate infection of another generation of mosquito larvae in the same way. Occlusion bodies are virus particles embedded in special protein lattice in order to maintain its viability in the environment after death of the insect host. It was reported that supplementation of salts, especially magnesium salts, significantly increased the level of infection.[28] Several favourable properties, such as thermal stability, ease of storage at $4^{O}C$, short generation time and highly infectious progeny viruses supports its development for mosquito control. Microencapsulation of viral occlusion bodies with magnesium has been proposed as a novel formulation which is likely to be a more effective in enhancing the infection level.[28]

CONCLUSION

The health hazards associated with chemical insecticides are well recognised. Use of entomopathogenic microorganisms is an eco-friendly, natural and effective approach. Although it's wider use is limited because of difficulty in large-scale production, storage and administration, lower infection rate, narrow range of susceptible mosquito host and lower mortality, entomopathogenic organisms being self-propagating in nature can significantly reduce the cost by obviating the need for repeated administration. It is likely to emerge as an essential tool for the control of mosquito and other vector borne diseases in near future

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